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TITLE: Disparities in Intratumoral Steroidogenesis

PRINCIPAL INVESTIGATOR: Stephen Freedland

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center
LOS ANGELES, CA 90048

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of these studies is to identify a biological origin underlying the racial disparity in prostate cancer incidence and mortality and to determine whether we can modulate this disparity by therapeutically targeting elevated cholesterol.

Prostate cancer disproportionately affects African American men; they are more than 1.5 times as likely then Caucasian men to develop prostate cancer and nearly 2.5 times as likely to die of prostate cancer. The reasons for this racial disparity in prostate cancer incidence and mortality are unknown but may stem from economic, social, psychological and biological origins. In the current proposal we hypothesize that one biological reason for the higher rates of prostate cancer incidence and mortality in African Americans is that the level of critical hormones that promote tumor growth (i.e. androgens e.g. testosterone) and the enzymes that form them are elevated in the prostate tumors of African American men. We further hypothesize that elevated cholesterol, which is an essential component of androgen formation, contributes to androgen formation in prostate tumors at a higher rate in African American vs. Caucasian men.

In this proposal we will examine the prostates of men with prostate cancer (50% African American, 50% Caucasian) after surgery to remove the prostate and determine whether there is a difference in testosterone and related androgens, as well as the enzymes that create them in the prostates of African American vs. Caucasian men.

The second aspect of this proposal is to determine whether we can reduce the levels of testosterone and related androgens and slow the growth of prostate tumors. We have developed animal models that permit us to determine the effects of elevated cholesterol on prostate tumor growth. Using these models we have been able to demonstrate that elevated cholesterol contributes to the formation of testosterone in prostate tumors of human origin. One aspect of our experimental approach is to test whether therapeutically targeting cholesterol using heart healthy diets and FDA approved, safe and effective drugs are able to reduce the formation of testosterone in prostate cancers.

The results of the research is designed to be immediately available to human patients as the use of FDA approved cholesterol lowering drugs and low fat, low cholesterol diets have few side effects or safety concerns and otherwise reduce the risk of cardiovascular disease, something that is generally desirable.

We anticipate that numerous men will be able to benefit from the research. Many men with cholesterol levels that do not put them at risk of cardiovascular disease may yet be at risk from the prostate cancer promotional effects of high cholesterol. Essentially, we anticipate the level of cholesterol reduction needed to protect the prostate will be somewhat lower than that to protect the heart. Thus, men that are at high risk of prostate cancer but have normal or slightly elevated levels of cholesterol may opt to reduce their cholesterol levels in order to reduce the risk of prostate cancer.

We also believe that this research will be especially helpful to African American men as they are less likely to use cholesterol lowering drugs than Caucasians. Increased usage of cholesterol lowering drugs by African American men may have a substantial effect at reducing the racial disparity in prostate cancer.

The ultimate goal of these studies is to provide a way to reduce prostate cancer risk and the risk of fatal prostate

15. SUBJECT TERMS

Nothing Listed

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1. INTRODUCTION

This was a Health Disparity Prostate Cancer Research Award originally to Dr. Keith Solomon at Boston Children's Hospital. In 2015, Dr. Solomon lost the rest of his funding and was let go from Boston Children's hospital. In May 2015, the grant with the remaining funds was transferred to Cedars-Sinai under the leadership of Dr. Stephen Freedland, who was appointed PI of the grant. Unfortunately, Dr. Freedland has been unable to get a hold of Dr. Solomon since 2015 and therefore has no knowledge about what was done prior to 2015. Therefore, this final report will include all work done at Cedars-Sinai since May 1, 2015.

The goal of these studies is to identify a biological origin underlying the racial disparity in prostate cancer incidence and mortality. Based upon very preliminary data, we generated the hypothesis that racial differences in how tumor cells handle cholesterol may under some of the racial differences seen in incidence and mortality. We sought to test this using preclinical models (done by Dr. Solomon for which no data are available) and using human prostate cancer samples (findings described below). Indeed, we have shown that there are racial differences in prostate cancer genotype and phenotype by race and appears to related to response to cholesterol. While the exact implications of these findings at this time are not 100% clear, we believe that overall goals of the award have been met and we have a further understanding of the biological origins of racial differences in prostate cancer.

2. KEY WORDS
Racial disparity
Prostate cancer
Radical prostatectomy
Gene expression
Cholesterol
Androgens

3. ACCOMPLISHMENTS
As noted above, the results described below covers the work done under the supervision of Dr. Stephen Freedland at Cedars-Sinai Medical Center. The key goals for Dr. Freedland were to understand the genetics of prostate cancer as a function of race and specifically examine how cholesterol homeostasis and androgen signaling correlate and perhaps differ by race. As the aims evolved over the course of the study (with prior approval by the DOD), we have broken activities below down by Task as approved in our most recent statement of work.

Task 1. Determine whether there is a disparity in the level of steroidogenic enzymes and androgens between the prostatic tumors of African American and Caucasian men (1-5 months). This task requires a bioinformatic analysis of pre-existing samples of men who underwent radical prostatectomy at the Durham VA along with serum cholesterol levels. The bioinformatic analysis will occur by the team at Cedars-Sinai Medical Center (CSMC) but using samples from the Durham VA.
1a. Finish gene expression analysis of samples from men treated by radical prostatectomy at the Durham VA – this is underway. Note no funds are requested for performing the gene expression – just the analysis of the data.

This has been completed. Results are presented below under tasks 1d and 1f. Of note, this involved detailed gene expression analysis of ~110 radical prostatectomy specimens. While we have performed profiling on ~620 tumors, only 110 were available for analysis at the time of this final study report.

1b. Abstract relevant data from the medical records of the patients including statin use and serum cholesterol levels.

This has been completed.

1c. Compile all data (gene expression, statin use, cholesterol, and race) into a master database.

This has been completed

1d. Analysis of androgen activity in the tumor.

This has been completed. Using the gene expression profile of 110 radical prostatectomy specimens from the Durham VA, we compared African-American (AA) and Caucasian American (CA) men on 'Androgen Biosynthesis' and 'Androgen Response' signatures which are obtained from the literature [1] and molecular signature database (http://software.broadinstitute.org/gsea/msigdb/collectio ns.jsp). The signature enrichment score was measured based on previously published method [2]. As seen in the figures below, there are no racial differences in androgen biosynthesis or androgen response gene signatures in the radical prostatectomies specimens by race.
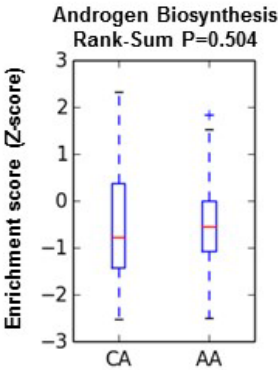

Figure 1. Androgen biosynthesis gene expression scores among African-American (AA) and Caucasian American (CA) men undergoing radical prostatectomy at the Durham VA.
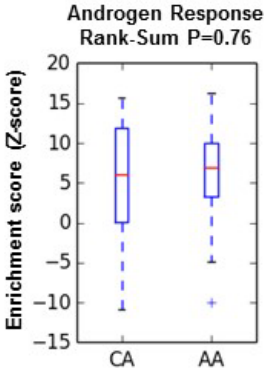
Figure 2. Androgen response gene expression scores among African-American (AA) and Caucasian American (CA) men undergoing radical prostatectomy at the Durham VA.

1f. Analysis of steroidogenic enzymes expression level

This has been completed. As seen in the figures below, there are significant racial differences in cholesterol biosynthesis, steroid biosynthesis, and cholesterol homeostasis gene signatures in the radical prostatectomies specimens by race. The lists of the genes involved in the signature were obtained from Reactome pathway database (https://reactome.org/) and KEGG (https://www.genome.jp/kegg/) pathway database.
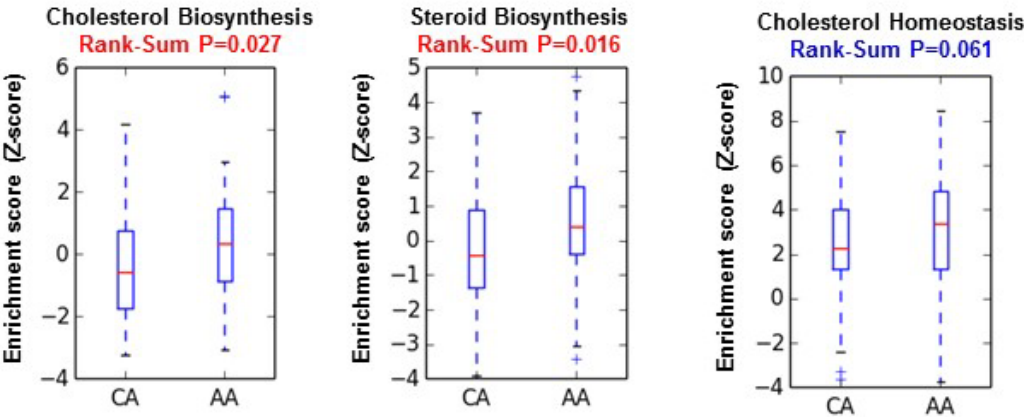

Figure 3. Gene expression scores for cholesterol biosynthesis, steroid biosynthesis, and cholesterol homeostasis among African-American (AA) and Caucasian American (CA) men undergoing radical prostatectomy at the Durham VA.

1g. Statistical analysis of the racial disparity in steroidogenesis and its association with cholesterol level.

This has been completed. Below we show the association between androgen response and cholesterol homeostasis separately by race. As can be seen, there is a positive and strong correlation between androgen response and cholesterol homeostasis gene signatures in the radical prostatectomies specimens in both races.
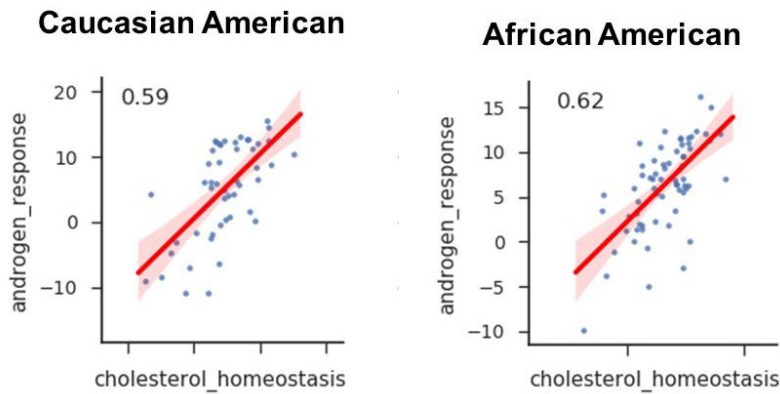
Figure 4. Correlation between gene expression scores for androgen response and cholesterol homeostasis among African-American (AA) and Caucasian American (CA) men undergoing radical prostatectomy at the Durham VA.

1h. Preparation of materials for data dissemination

This has been completed with completion of this grant report. The publication from these data is in preparation.

References:

1.   Stuchbery, R., et al., *Androgen synthesis in prostate cancer: do all roads lead to Rome?* Nat Rev Urol, 2017. **14**(1): p. 49-58.
2.   You, S., et al., *Integrated Classification of Prostate Cancer Reveals a Novel Luminal Subtype with Poor Outcome.* Cancer Res, 2016. **76**(17): p. 4948-58.

4. IMPACT

The underlying goal of our study was to understand racial differences in prostate cancer biology. Toward this end, we have identified key differences in cholesterol biology by race. Identifying differences is the first step in ultimately developing novel treatments. Though no specific treatment has emerged from our work to date, its impact lies in the fact that we now know where to focus our energies: understanding the racial differences in cholesterol metabolism. Of note, we have separate DOD funding that is just starting to test novel cholesterol-targeted treatments for prostate cancer. Thus, it is hoped that the findings from the current now finished DOD grant can be combined with the knowledge from our upcoming DOD grant to identifying not only optimal cholesterol-targeting approaches, but in which patients and which races these treatments will be most impactful.

5. CHANGES/PROBLEMS

During the course of the study, we identified several challenges, which necessitated a change in study plans. Of note, all study plan changes were approved in advance by the DOD.

1. Change in study recruitment

    Initially, we intended to prospectively recruit men from Duke University and the Durham VA Hospital. However, due to several unforeseen issues, we early on abandoned the idea of recruitment at Duke University. This was both a budgetary issue (costs were too high), a logistical issue (recruitment was very slow and challenging), and a scientific issue (the number of black men was low). Therefore, we continued enrollment at the Durham VA only.

2. Change in PI

   As noted above, in 2015, Dr. Solomon lost the rest of his funding and was let go from Boston Children's hospital. In May 2015, the grant with the remaining funds was transferred to Cedars-Sinai under the leadership of Dr. Stephen Freedland, who was appointed PI of the grant. Unfortunately, Dr. Freedland has been unable to get a hold of Dr. Solomon since 2015 and therefore has no knowledge about what was done prior to 2015. As such, this change not only involved the loss of key data from Boston Children's, but also a slowdown in enrollment and delays for Dr. Freedland.

3. Further change in study plans

   Given the delays getting the transfer to Cedars-Sinai and our inability due to lack of DOD approval to enroll patients during the change in PI, and the time cap on this award, we were unable to prospectively enroll sufficient numbers of patients. As such, what was intended for prospective enrollment, resulted in being an analysis of pre-existing samples. With improvement in human genomic analysis during the course of the award, coupled with a strong pre-existing collaboration Dr. Freedland had with GenomeDx, for the same funds we were able to analyze a larger sample of patients more comprehensively than originally planned.

6. PRODUCTS

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: Stephen Freedland
Project Role: PI
Researcher identifier (ORCID ID): 0000-0002-8104-6419
Nearest person month worked: 1
Contribution to project: Overall all work performed

Name: Michael Freeman
Project Role: co-I
Researcher identifier (ORCID ID): Unknown
Nearest person month worked: 1
Contribution to project: Helped with the interpretation of the genomic data

Name: Sungyong You
Project Role: co-I
Researcher identifier (ORCID ID): 0000-0003-3513-1783
Nearest person month worked: 1
Contribution to project: Performed the gene expression analyses

Name: Adriana Vidal
Project Role: co-I
Researcher identifier (ORCID ID): Unknown
Nearest person month worked: 2
Contribution to project: Helped with the interpretation of the genomic data

Name: Shweta Dambal
Project Role: post-doc
Researcher identifier (ORCID ID): Unknown

Nearest person month worked: 12
Contribution to project: Helped with the interpretation of the genomic data; helped analyze the data

Name: Sergio Sanders
Project Role: technician
Researcher identifier (ORCID ID): Unknown
Nearest person month worked: 12
Contribution to project: Helped pull samples and send for analysis

Name: Erick Maravilla
Project Role: technician
Researcher identifier (ORCID ID): Unknown
Nearest person month worked: 8
Contribution to project: Helped pull samples and send for analysis

Name: Dana Levin
Project Role: research coordinator
Researcher identifier (ORCID ID): Unknown
Nearest person month worked: 8
Contribution to project: Coordinated pulling samples and sending for analysis; ensured all IRB protocols were in place and all work was done in a HIPAA compliant manner

What other organizations were involved as partners?
  Organization name: Durham VA Medical Center
  Location of organization: Durham, NC
  Partner's contribution to the project: providing samples for analysis

9. SPECIAL REPORTING REQUIRMENTS

N/A

10. APPENDICES

N/A